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Authors

Miao, Guanhong

Pechlaner, Raimund

Malloy, Kimberly

et al.

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ORIGINAL RESEARCH

Longitudinal Lipidomic Signature of Coronary Heart Disease in American Indian People

Guanhong Miao, PhD; Raimund Pechlaner, MD; Oliver Fiehn, PhD; Kimberly M. Malloy, MS; Ying Zhang, MD, PhD; Jason G. Umans, MD; Manuel Mayr, MD, PhD; Johann Willeit, MD; Stefan Kiechl, MD; Jinying Zhao, MD, PhD

BACKGROUND: Dyslipidemia is an independent risk factor for coronary heart disease (CHD). Standard lipid panel cannot capture the complexity of the blood lipidome (ie, all molecular lipids in the blood). To date, very few large-scale epidemiological studies have assessed the full spectrum of the blood lipidome on risk of CHD, especially in a longitudinal setting.

METHODS AND RESULTS: Using an untargeted liquid chromatography–mass spectrometry, we repeatedly measured 1542 lipid species from 1835 unique American Indian participants who attended 2 clinical visits (≈5.5 years apart) and followed up to 17.8 years in the Strong Heart Family Study (SHFS). We first identified baseline lipid species associated with risk of CHD, followed by replication in a European population. The model adjusted for age, sex, body mass index, smoking, hypertension, diabetes, low-density lipoprotein cholesterol, estimated glomerular filtration rate, education, and physical activity at baseline. We then examined the longitudinal association between changes in lipid species and changes in cardiovascular risk factors during follow-up. Multiple testing was controlled by the false discovery rate. We found that baseline levels of multiple lipid species (eg, phosphatidylcholines, phosphatidylethanolamines, and ceramides) were associated with the risk of CHD and improved the prediction accuracy over conventional risk factors in American Indian people. Some identified lipids in American Indian people were replicated in European people. Longitudinal changes in multiple lipid species (eg, acylcarnitines, phosphatidylcholines, and triacylglycerols) were associated with changes in cardiovascular risk factors.

CONCLUSIONS: Baseline plasma lipids and their longitudinal changes over time are associated with risk of CHD. These findings provide novel insights into the role of dyslipidemia in CHD.

Key Words: American Indian ■ cardiovascular disease ■ coronary heart disease ■ longitudinal lipidomics ■ Strong Heart Study

Cardiovascular disease (CVD) is the leading cause of death worldwide, and coronary heart disease (CHD) accounts for the greatest proportion of CVD.[1] American Indian people have higher risks for CVD, diabetes, and other risk factors.[1] Dyslipidemia, defined as a high level of low-density lipoprotein cholesterol (LDL-c), triglycerides, or total cholesterol, or a low level of high-density lipoprotein cholesterol (HDL-c), is the major risk factor for CVD.[2,3] Standard lipid panel that measures bulk lipoproteins cannot reflect the diverse classes of individual molecular lipid species in the blood (ie, blood lipidome), and thus has limited value in detecting the early disturbance of lipid metabolism implicated in CVD.

Lipidomics is a mass spectrometry–based omics technology that can simultaneously identify and quantify many individual lipid species in a biological sample. Using this technology, several epidemiological studies

Correspondence to: Jinying Zhao, MD, PhD, Center for Genetic Epidemiology and Bioinformatics, University of Florida, Department of Epidemiology, College of Public Health and Health Professions and College of Medicine, University of Florida, 2004 Mowry Rd, Gainesville, FL 32610. Email: jzhao66@ufl.edu

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CLINICAL PERSPECTIVE

What Is New?

- This is the first longitudinal lipidomic study of coronary heart disease (CHD) in a large community-based prospective cohort of American Indian people, followed by replication in European people in an independent population study.
- Altered baseline levels of multiple individual lipid species were associated with risk of CHD in American Indian participants, and the top identified lipids significantly improved risk prediction for CHD beyond traditional risk factors.
- Longitudinal changes in multiple individual lipid species were associated with changes in CHD risk factors.

What Are the Clinical Implications?

- Identifying novel molecular lipid species not only deepens our understanding of the mechanism through which dyslipidemia contributes to CHD, but also provides novel biomarkers for risk stratification and early detection of CHD.
- If validated, the identified lipid species may serve as novel biomarkers for risk stratification and early prediction of CHD.

Nonstandard Abbreviations and Acronyms

AC	acylcarnitine
CE	cholesterol ester
CER	ceramide
DAG	diacylglycerol
FDR	false discovery rate
GlcCer	glucosylceramide
PC	phosphatidylcholine
PE	phosphatidylethanolamine
SM	sphingomyelin
SHFS	Strong Heart Family Study
TAG	triacylglycerol

have identified associations of altered lipid species, eg, triacylglycerols (TAGs), ceramides (CERs), phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), and sphingomyelins (SMs) with CVD[4–8] and its risk factors[9,10] in human populations. However, previous studies were largely cross-sectional or limited by small sample size, and/or low coverage of the blood lipidome. We are unaware of large-scale longitudinal lipidomic studies that examined the relationship between changes in individual lipid species and the risk of CHD in a well-characterized prospective cohort of any racial and ethnic group.

Leveraging the longitudinal lipidomic data in 3595 fasting blood samples from 1835 unique American Indian individuals who attended 2 clinical examinations (mean follow-up: 5.5 years) and were free of overt CVD at baseline in the SHFS (Strong Heart Family Study), we conducted the first large-scale longitudinal lipidomic profiling of CHD in American Indian people. Putative lipids identified in American Indian people were replicated in European White people in the Bruneck Study. Our primary goals are to (1) identify individual lipid species associated with risk of CHD beyond known risk factors, and (2) examine the longitudinal associations between changes in plasma lipidome and changes in cardiometabolic traits in American Indian people.

METHODS

The phenotype data used in the SHFS can be requested through the SHS (Strong Heart Study) (https://strongheartstudy.org/). The lipidomic data in the SHS can be obtained from the corresponding author upon a reasonable request. Data of the Bruneck Study are available upon reasonable request to the Bruneck data-sharing committee.

Study Populations

The SHFS (2001-ongoing) is a family-based prospective study designed to identify genetic and lifestyle factors for CVD and its risk factors in American Indian people.[11] Briefly, 2786 tribal members (aged ≥14 years) in 3 geographic regions (Arizona, North Dakota, South Dakota, and Oklahoma) were initially examined in 2001 to 2003 and re-examined after 5-year follow-up (2006–2009) using the same protocols. Information for demography, lifestyle, family history, and medical records was collected at each visit. Detailed methods for the study design, laboratory protocols, and phenotype collection were reported previously.[11,12] The current analysis included 1835 individuals (mean age at baseline: 39.8 years) who were free of overt CVD at baseline, had longitudinal lipidomic data at 2 time points (2001–2003 and 2006–2009, mean 5.5 years apart), and complete information for clinical and follow-up data until December 31, 2020. The mean follow-up period is 17.8 years (range 0–19.9 years). All participants provided informed consents. The SHFS protocols were approved by the Institutional Review Boards of the participating institutions and the American Indian tribes.

The Bruneck Study is a population-based prospective survey on the epidemiology and pathogenesis of atherosclerosis and CVD.[4] In 1990, the study enrolled and examined a random sample of inhabitants residing

in the Bruneck area, Italy (n=1000).[4,13,14] Follow-up assessments were performed every 5 years thereafter. The present study included 637 individuals who were free of overt CVD at baseline and have lipidomic data (collected in 2000) and subsequent clinical follow-up data until 2010. The study protocol was approved by the ethics committees of Bolzano and Verona and conformed to the Declaration of Helsinki, and all study subjects gave their written informed consent.[4]

Ascertainment of CVD Events

In the SHFS, baseline information was collected in 2001 to 2003 and living participants were followed through December 31, 2020. CHD included definite CHD (fatal or nonfatal), definite myocardial infarction (fatal or nonfatal), and sudden death due to CHD.[15,16] More details for the definitions of fatal and nonfatal events have been described previously.[15,16] Briefly, CHD events that occurred during the follow-up period were ascertained by annual mortality and morbidity survey. In the annual surveillance, participants were contacted to determine their vital status and, if living, whether any cardiovascular events of interest had occurred since last contact. Medical records were abstracted and CHD death and events were ascertained and confirmed by the mortality and morbidity review committees using specific criteria. Time to event was calculated from the date of baseline examination (2001–2003) to the date of CHD event or the last follow-up. If a participant experienced >1 CHD event during the follow-up period, the earliest date was used as the date of the event.

In the Bruneck study, detailed information about fatal and nonfatal new-onset CVD was collected during the follow-up period between 2000 and 2010.[4] A composite CVD end point included incident fatal and nonfatal myocardial infarction, ischemic stroke, and sudden cardiac death.[4] The presence of myocardial infarction was assessed by World Health Organization criteria, and ischemic stroke was classified according to the criteria of the National Survey of Stroke. Ascertainment of CVD events or procedures did not rely on hospital discharge codes or the patient's self-report information but on a careful review of medical records provided by the general practitioners and files of the Bruneck Hospital and the extensive clinical and laboratory examinations performed as part of the study protocols.[13,14]

Assessment of Covariates

In the SHFS, fasting plasma glucose, insulin, lipoproteins, and creatinine were measured by standard laboratory methods as previously described.[12] Anthropometric measurements including body weight, height, and waist circumference were measured with participants wearing light clothing and without shoes by trained research staff. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters. Information regarding previous/current medical conditions, education, smoking, and family history of diseases was collected during the personal interview. Based on years of education, participants were grouped into 2 classes (ie, those who have <12 years of education or ≥12 years of education. For cigarette smoking, participants were classified as current smokers and noncurrent smokers (former and never smokers combined). Hypertension was defined as blood pressure ≥130/80 mm Hg[17] or use of antihypertensive medications. Type 2 diabetes was defined as fasting plasma glucose ≥126 mg/dL or use of hypoglycemic drugs. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration 2021 creatinine-based equation.[18] Physical activity was assessed by the mean number of steps per day calculated by averaging the total number of steps recorded each day during the 7-day period. Information on use of lipid-lowering drugs was also collected at each visit.

In the Bruneck study, information on lifestyle and clinical factors, such as BMI, current smoking, blood pressure, clinical lipids, and blood glucose, was obtained as previously described.[4]

Lipidomic Data Acquisition, Preprocessing, and Quality Control

In the SHFS, relative abundance of molecular lipid species in fasting plasma samples at 2 time points (≈5.5 years apart) was quantified by untargeted liquid chromatography mass spectrometry.[19] Detailed methods for lipidomic data acquisition and preprocessing have been described elsewhere.[19] After preprocessing and quality control, we obtained 1542 lipids (518 known) in 3977 samples (1983 at baseline, 1994 at follow-up). After further excluding individuals with prevalent CVD or those with missing covariates, a total of 3595 plasma samples (1835 at baseline, 1760 at follow-up, mean 5.5 years apart) from 1844 unique participants were included in the current analysis.

Lipidomic profiling in the Bruneck Study was performed using mass spectrometry as previously described.[4] Of the 518 known lipids measured in the SHFS, 102 lipids were also measured in 637 individuals in the Bruneck, and these lipids were used in the replication analysis.

Statistical Analysis

The procedures for participants' selection are illustrated in Figure S1. Figure 1 shows the workflow of the study. All continuous variables including lipids were standardized to zero mean and unit variance.

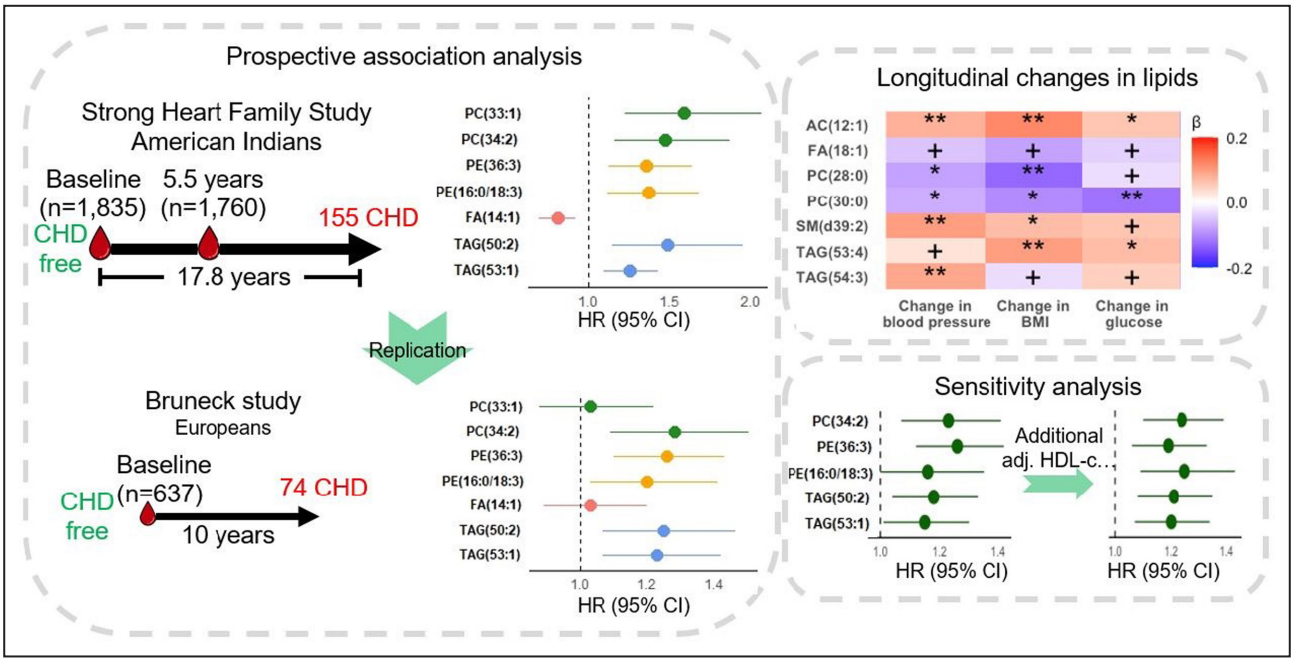

Figure 1. Workflow of the study.
AC indicates acylcarnitine; BMI, body mass index; CHD, coronary heart disease; FA, fatty acid; HDL-c, high-density lipoprotein cholesterol; HR, hazard ratio; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; and TAG, triacylglycerol. +, $P<0.05$; *, $q<0.05$; **, $q<0.01$.

Prospective Association Analysis

To identify baseline plasma lipid species associated with risk of CHD, we constructed frailty Cox proportional hazards models in the SHFS. In this model, time to CHD onset was the dependent variable and the baseline level of each lipid was the independent variable. The model adjusted for age, sex, BMI, smoking, hypertension, diabetes, LDL-c, eGFR, education, and physical activity at baseline. The frailty term was used here to account for the relatedness among family members. Multiple testing was controlled by false discovery rate using the Storey's q-value method.[20,21] Lipids with $P<0.05$ in the SHFS were then replicated in the Bruneck study, adjusting for age, sex, BMI, smoking, hypertension, diabetes, LDL-c, and eGFR at baseline. Replication was defined as lipids with $P<0.05$ and consistent directions of association in both studies. Results from 2 cohorts were then combined by fixed-effects meta-analysis.

To further assess the potential incremental prognostic value of the identified lipid species for CHD prediction over traditional risk factors, we split the entire SHFS sample into 2 sets: a training set that included participants from Dakota and Arizona (n=1038, 82 cases), and a testing set that included participants from Oklahoma (n=797, 73 cases). We compared a base model including risk factors only and a model containing both risk factors and the identified lipid species ($q<0.05$). The incremental prognostic value of the identified lipids over traditional risk factors was assessed by the area under the receiver operating characteristic curve.[22]

Repeated Measurement Analysis

Of the 1760 participants free of CVD at both baseline and follow-up (mean 5.5 years apart), we examined the association between changes in lipid species (between baseline and follow-up) and changes in cardiometabolic traits (ie, BMI, waist circumference, blood pressure, glucose, insulin, insulin resistance, and eGFR). To achieve this, we constructed a generalized estimating equation model, in which the percent change in each cardiometabolic trait (ie, the difference in a measurement between follow-up and baseline, divided by the baseline) was the outcome and percent change in the relative abundance of an individual lipid species was the predictor. The model adjusted for age, sex, smoking, obesity, hypertension, diabetes, LDL-c, education, and physical activity at baseline. This analysis was performed in the SHFS only because the Bruneck study only measured blood lipid species at baseline.

Sensitivity Analysis

To examine whether clinical lipids (eg, HDL-c, total cholesterol) and use of lipid-lowering drugs affect our results, we conducted sensitivity analyses by additionally

adjusting for these variables in the above-described prospective analyses. This analysis focused on significant lipids in the SHFS.

RESULTS

The mean age of participants at baseline was 39.8 and 65.3 years old in the SHFS and Bruneck, respectively. Over an average of 17.8-year follow-up in the SHFS, 155 participants experienced incident CHD, resulting in an incidence rate of 4.8 (95% CI, 4.0–5.6) per 1000 person-years. In the Bruneck study, 74 participants developed incident CVD events over a 10-year follow-up period and the incidence rate is 13.7 (95% CI, 10.7–17.1) per 1000 person-years. The Table presents baseline characteristics of participants in the SHFS. Compared with American Indian participants who did not develop incident CHD (noncases), those who developed incident CHD (cases) were significantly older, more likely to be men, more likely to have hypertension and diabetes, and had higher levels of systolic blood pressure, total cholesterol, LDL-c, triglycerides, and fasting glucose but lower levels of eGFR and physical activity at baseline. Similar patterns were also observed in the Bruneck study (Table).

Baseline Plasma Lipid Species Predict Risk of CHD Beyond Traditional Risk Factors

In the SHFS, baseline levels of 92 lipid species (out of 518 known lipids) were significantly associated with incident CHD at $P<0.05$ (Table S1). Specifically, higher baseline levels of 81 lipid species, including 28 phosphatidylcholines (PCs), 22 phosphatidylethanolamines (PEs), 13 sphingomyelins (SMs), 9 ceramides (CERs), 5 TAGs, 2 phosphatidylglycerols (PGs), 1 cholesterol ester (CE), and 1 glucosylceramide, were associated with an increased risk of CHD. In contrast, higher baseline levels of 10 fatty acids (FAs) and cholesterol were associated with a decreased risk of CHD. After correction for multiple testing, baseline levels of 10 lipids remained to be significantly associated with incident CHD at $q<0.05$ (Figure 2). Of these 10 lipids, higher baseline levels of 9 lipids, including 3 CERs (ie, CER(d40:1), CER(d40:2), CER(d41:1)), 3 PCs (ie, PC(38:5) B, PC(40:7) A, PC(p-38:2)/PC(o-38:3)), 2 PEs (ie, PE(16:0/22:5), PE(p-38:3)/PE(o-38:4)), and 1 SM (ie, SM(d40:1) B), were significantly associated with an increased risk of CHD. In contrast, higher baseline level of 1 long-chain unsaturated FA (ie, FA(14:1)) was significantly associated with a decreased risk of CHD. Additional inclusion of these 10 lipids ($q<0.05$) significantly improved CHD risk prediction over traditional

Table. Baseline Characteristics of Participants in 2 Cohorts According to the Status of Incident Coronary Heart Disease (CHD) or Cardiovascular Disease (CVD) by End of Follow-Up (Follow-up: Mean 17.8 Years in the SHFS and 10 Years in the Bruneck Study)

	SHFS			Bruneck		
Characteristics	CHD (n=155)	Noncases (n=1680)	*P* value	CVD (n=74)	Noncases (n=563)	*P* value
Age, y	52.1±12.2	38.7±13.5	$<2.0\times10^{-16}$	71.6±10.5	64.4±9.6	2.42×10^{-8}
Female, n (%)	79 (51.0%)	1071 (63.7%)	0.002	34 (45.9%)	303 (53.8%)	0.203
BMI, kg/m^2	32.4±6.4	31.7±7.6	0.188	26.6±4.4	25.3±3.9	0.012
Current smoking, n (%)	58 (37.4%)	670 (39.9%)	0.561	12 (16.2%)	95 (16.9%)	0.887
Hypertension, n (%)	111 (71.6%)	857 (51.0%)	1.12×10^{-6}	55 (74.3%)	300 (53.3%)	8.51×10^{-4}
Type 2 diabetes, n (%)	78 (50.3%)	240 (14.3%)	$<2.0\times10^{-16}$	13 (17.6%)	46 (8.2%)	0.011
Systolic blood pressure, mm Hg	131.0±16.3	121.2±14.9	1.60×10^{-14}	144.9±19.3	138.6±18.1	0.006
Diastolic blood pressure, mm Hg	78.0±10.9	77.0±10.6	0.344	84.0±7.5	84.1±8.3	0.931
Total cholesterol, mg/dL	195.9±34.8	184.2±34.0	6.94×10^{-6}	242.6±50.3	232.9±40.8	0.063
HDL-c, mg/dL	50.2±13.0	52.2±14.6	0.077	55.0±14.7	58.0±15.4	0.112
LDL-c, mg/dL	107.7±29.7	101.1±29.5	0.004	155.5±43.9	148.1±35.6	0.101
Triglycerides, mg/dL	195.7±117.5	160.2±115.3	0.043	159.7±89.0	133.8±72.8	0.007
Fasting glucose, mg/dL	135.9±65.8	106.0±42.2	5.43×10^{-12}	109.3±34.6	99.8±22.0	0.003
eGFR, mL/min per 1.73 m^2	110.7±20.0	124.5±15.7	1.11×10^{-16}	77.9±17.9	83.0±13.4	0.004
Education (<12 years), n (%)	86 (55.5%)	1038 (61.8%)	0.128	—	—	—
Physical activity, steps/d	4844.0±3765.7	5919.2±3734.7	0.009	—	—	—

Continuous variables are expressed as mean±SD, and qualitative variables as n (%). *P* values were obtained by generalized estimating equation model accounting for correlation among family members. BMI indicates body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; and SHFS, Strong Heart Family Study. Cases: participants who developed incident CHD (SHFS) or CVD (Bruneck) by end of follow-up; non-cases: participants who did not develop incident CHD (SHFS) or CVD (Bruneck) by end of follow-up. Information for education and physical activity is not available in the Bruneck study.

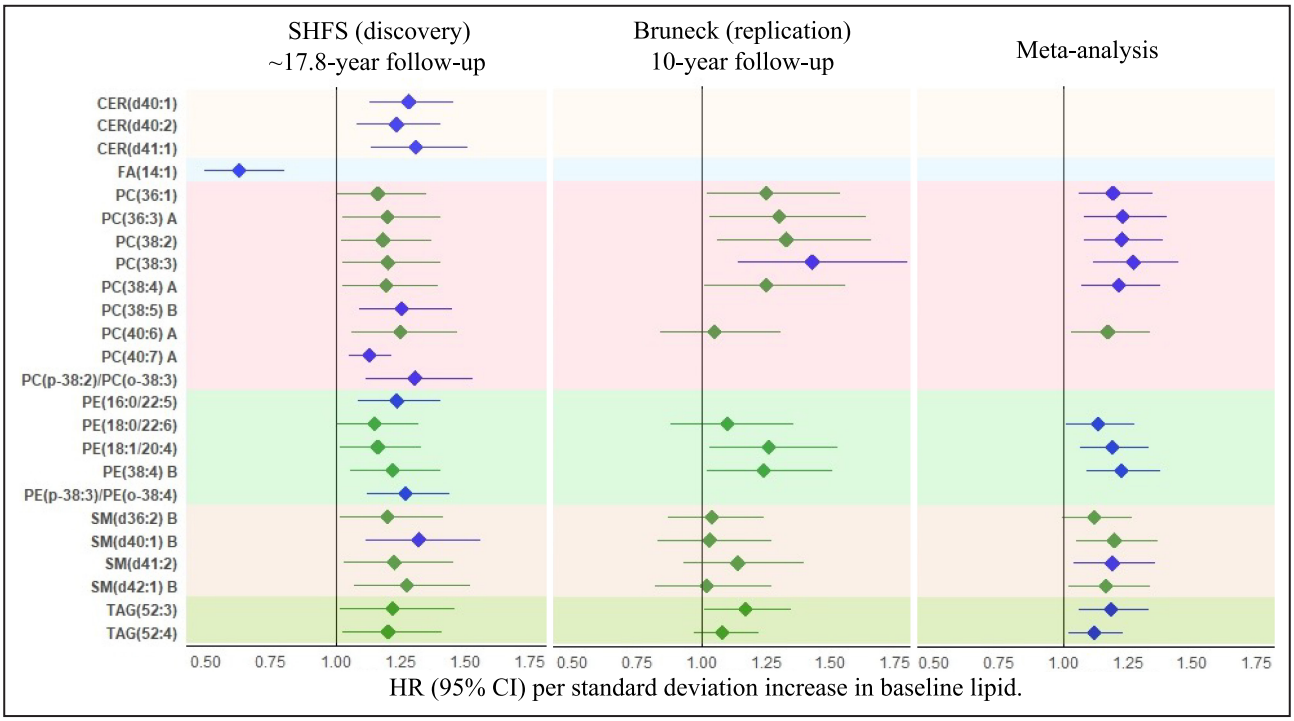

Figure 2. Baseline plasma lipid species significantly associated with risk of CHD.
Hazard ratios (HRs) and 95% CIs were obtained by Cox proportional hazards regression, adjusting for age, sex, body mass index, smoking, hypertension, diabetes, low-density lipoprotein cholesterol, estimated glomerular filtration rate, education, and physical activity at baseline. Family relatedness in the SHFS was accounted for by including a frailty term in the Cox model. Only lipids with $P<0.05$ are shown. Lipids highlighted in blue indicate $q<0.05$. The letters A and B in the lipid names represent isomers. CER indicates ceramide; CHD, coronary heart disease; FA, fatty acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SHFS, Strong Heart Family Study; SM, sphingomyelin; and TAG, triacylglycerol.

risk factors in American Indian participants (area under the receiver operating characteristic curve increased from 0.801 to 0.832, $P=0.013$; Figure 3).

Of the 92 known lipids associated with incident CHD in the SHFS ($P<0.05$), 15 lipids were also measured in the Bruneck study. Among these, 8 lipids, including 5 PCs (ie, PC(36:1), PC(36:3) A, PC(38:2), PC(38:3), PC(38:4) A), 2 PEs (ie, PE(18:1/20:4), PE(38:4) B) and TAG(52:3), were confirmed in the Bruneck study with the same direction of association ($P<0.05$; Figure 2, Table S2).

Transethnic meta-analysis identified 11 lipids significantly associated with risk of CHD ($q<0.05$) in both studies. Specifically, higher baseline levels of 5 PCs, 3 PEs, 2 TAGs, and 1 SM were associated with an increased risk of CHD (Figure 2, Table S2). To intuitively interpret our results, we summarized the observed associations and potential biological pathways in Figure S2.

Longitudinal Changes in Lipid Species Associated With Changes in Cardiometabolic Traits

After multiple testing correction ($q<0.05$), longitudinal changes in 1228 lipids (421 known) were significantly associated with changes in 1 or more cardiovascular risk factors (Figure 4; Table S3). Specifically, changes in ACs were inversely associated with changes in insulin resistance and eGFR, but positively associated with change in blood pressure. Changes in TAGs were positively associated with changes in fasting glucose, insulin resistance, BMI, and waist circumference, but inversely associated with change in blood pressure. Changes in FAs showed the most significant and inverse associations with change in insulin resistance. Changes in multiple lipid species of other lipid classes (eg, PCs, PEs, phosphatidylinositols, and SMs) were also positively or inversely associated with changes in cardiometabolic traits.

Sensitivity Analysis

Additional adjustments for clinical lipids (eg, HDL-c and total cholesterol) and use of lipid-lowering drugs did not change the associations between significant lipids and incident CHD (Table S4).

DISCUSSION

In this large-scale lipidomic profiling comprising ≈2500 individuals participating in 2 community-dwelling

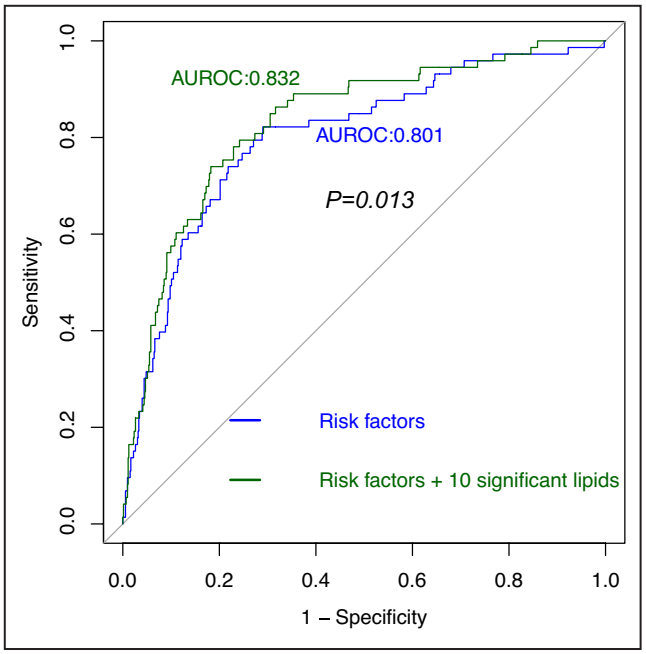

Figure 3. The identified plasma lipid species significantly increased risk prediction for CHD.
Model 1: traditional risk factors, including age, sex, BMI, smoking, hypertension, diabetes, LDL-c, eGFR, education, and physical activity at baseline. Model 2: traditional risk factors +10 significant lipids, including CER(d40:1), CER(d40:2), CER(d41:1), FA(14:1), PC(38:5) B, PC(40:7) A, PC(p-38:2)/PC(o-38:3), PE(16:0/22:5), PE(p-38:3)/PE(o-38:4), and SM(d40:1) B. Participants from the Dakota and Arizona Centers were used as the training set (n=1038, 82 cases), and those from the Oklahoma Center were used as the testing set (n=797, 73 cases). Compared with the model that included traditional risk factors only (model 1), inclusion of the identified lipids (model 2) significantly increased risk prediction for CHD (AUROC increased from 0.801 to 0.832, *P*=0.013). AUROC indicates area under the receiver operating characteristic curve; BMI, body mass index; CER, ceramide; CHD, coronary heart disease; eGFR, estimated glomerular filtration rate; FA, fatty acid; LDL-c, low-density lipoprotein cholesterol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; and SM, sphingomyelin.

prospective cohorts, we had several key findings. First, we found that baseline levels of multiple molecular lipid species (eg, PCs, PEs, and CERs) were significantly associated with risk of CHD beyond traditional risk factors. Some of the identified lipids were replicated in European White persons. Second, our repeated measurement analysis identified that longitudinal changes in multiple lipid species (eg,, ACs, PCs, and TAGs) were associated with changes in multiple cardiometabolic traits. Together, our results identified novel individual lipid species and distinct lipidomic signatures associated with risk for CHD. The identified lipid species provide novel insights into the role of dyslipidemia in CHD, and if validated, offer potential novel biomarkers for risk stratification, and early identification or prevention of CHD.

We found that elevated baseline levels of multiple PCs and PEs, such as PC(40:7) A, PC(38:3), PE(16:0/22:5), and PE(18:1/20:4), were associated with an increased risk of cardiovascular events in American Indian or European White people. Longitudinal changes in PCs and PEs were also significantly associated with changes in cardiometabolic traits in American Indians. These findings corroborate previous studies demonstrating that some PCs and PEs, such as, PC(32:0), PC(16:0/22:5), PC(14:0/22:6), PE(o-36:4), and PE(38:2), were associated with CVD in other racial and ethnic groups.[5,6,8,23] In addition, some PCs and PEs (eg, PC(36:1), PC(38:3), PC(36:3) A, and PE(16:0/22:5)) identified in the present study were also associated with risks of diabetes,[19] chronic kidney disease,[24] depressive symptoms,[25] or all-cause mortality[26] in our previous studies of the same group of American Indian participants. Moreover, these results support previous studies showing that altered levels of PCs in either plasma or atherosclerotic plaque tissues were associated with coronary atherosclerosis.[10,27] PCs and PEs are the most abundant phospholipids in mammalian cell membrane. They play important roles in numerous biological processes, such as energy metabolism, cell proliferation, adhesion, autophagy, stress responses, and apoptosis.[28] In addition, PCs can be hydrolyzed via phospholipase A2 (PLA2), which may promote vascular inflammation and oxidative stress, thereby contributing to plaque formation, rupture, and subsequent atherosclerotic CVD.[10,27]

Besides glycerophospholipids, altered baseline levels of sphingolipids (eg, CERs and SMs) and long-chain FAs as well as their longitudinal changes were also associated with the development of CHD in American Indian people. In support of previous studies,[7,23] we observed significant associations of altered plasma sphingolipids (eg, CER(d40:1), CER(d41:1), and SM(d40:1) B) with risk of CHD in American Indian people. Sphingomyelinase (SMase) catalyzes the hydrolysis of SMs to CERs and choline phosphate. Altered SMase activity has been involved in many biological pathways such as inflammation, oxidative stress, apoptosis, and atherogenesis,[29] all of which may contribute to CHD. In agreement with previous studies reporting the beneficial effects of unsaturated FAs on cardiovascular health,[30] we found that higher baseline levels of long-chain unsaturated FAs were associated with a decreased risk of CHD. This may be attributed to the protective effects of unsaturated FAs on cardiovascular health.[30]

Our repeated measurement analysis revealed, for the first time, the temporal relationship between change in plasma lipidome and change in cardiometabolic traits in American Indian people and individuals of other racial and ethnic groups. Besides the contributions of the above-mentioned lipids (eg, PCs, PEs, CERs,

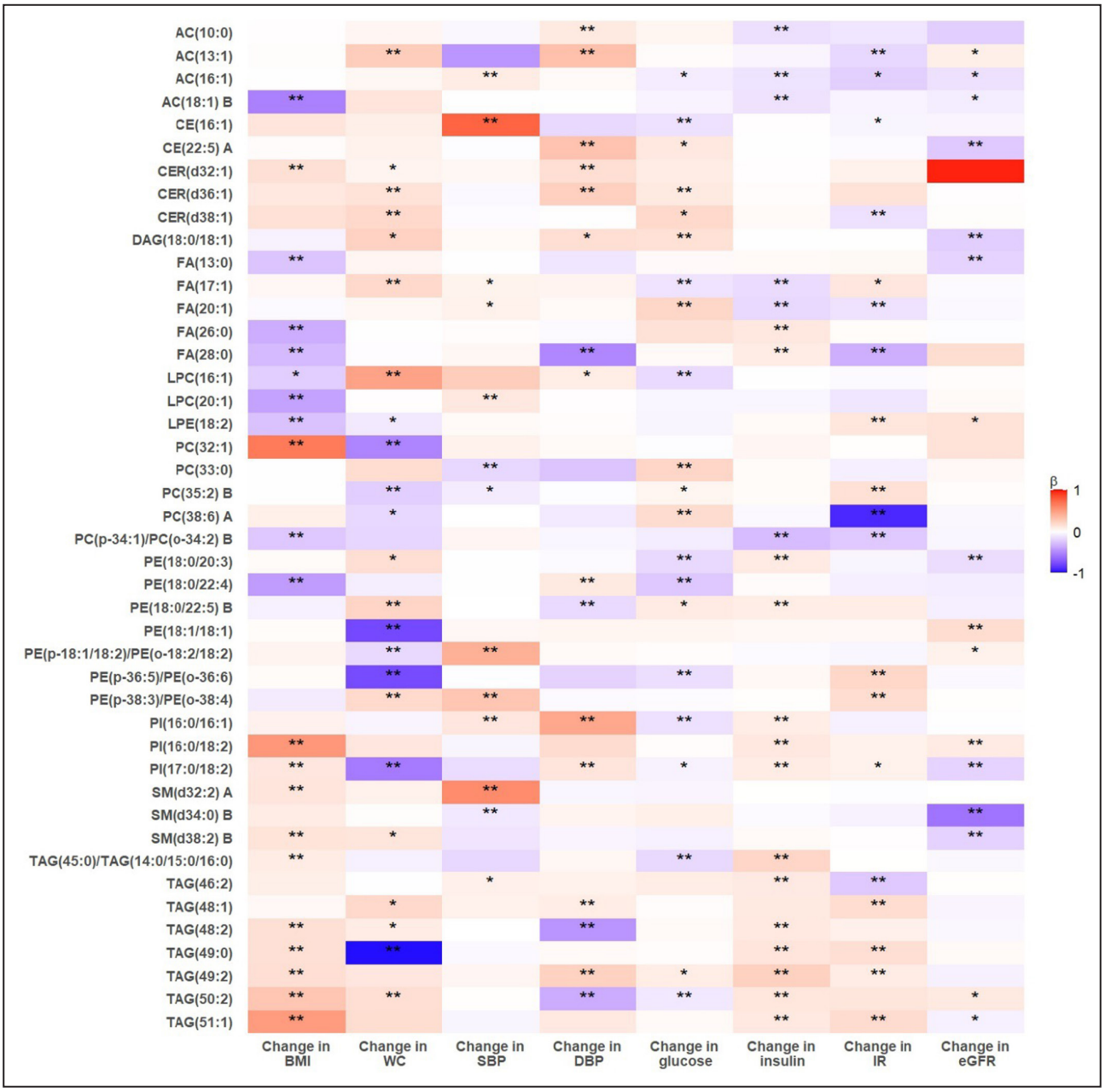

Figure 4. Heatmap showing the associations between longitudinal changes in plasma lipid species and changes in cardiovascular risk factors (mean 5.5 years apart) among American Indian participants in the SHFS.
Only top-ranked lipids are shown. Color codes are based on regression coefficients (β) derived from the generalized estimating equation model, adjusting for age, sex, obesity status, smoking, hypertension, diabetes, LDL-c, education, and physical activity at baseline. The letters A and B in the lipid names represent isomers. AC indicates acylcarnitine; BMI, body mass index; CE, cholesterol ester; CER, ceramide; DAG, diacylglycerol; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FA, fatty acid; IR, insulin resistance; LDL-c, low-density lipoprotein cholesterol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SBP, systolic blood pressure; SHFS, Strong Heart Family Study; SM, sphingomyelin; TAG, triacylglycerol; and WC, waist circumference. **q<0.05, *P<0.05.

SMs, and FAs) to the development of CHD, changes in several lipid classes, such as TAGs, phosphatidylinositols, and ACs, were also associated with changes in multiple cardiometabolic traits. It is likely that elevated levels of TAGs may cause atherosclerotic lesions through promoting vascular inflammation, endothelial dysfunction, and procoagulant activities.[31] Arachidonic acids, which can be derived from phosphatidylinositol via phospholipase A2,[10] are known to be involved in inflammation and coagulation, both of which are implicated in atherosclerotic CVD.[32] ACs may contribute to atherosclerotic CVD through their roles in multiple

biological processes, such as cardiac function, inflammation, mitochondrial β-oxidation, energy metabolism, cellular stress, and insulin signaling.[33] Perturbed ACs have been associated with a wide range of metabolic disorders including CVD,[34] diabetes,[35] and depressive symptoms.[25]

Several limitations of our study should be noted. First, although our high-resolution liquid chromatography mass spectrometry detected 1542 lipid species in our samples, many lipids are unknown, and isomeric lipids cannot be determined either. Additional experiments are needed to characterize these unknowns if considered of interest. Moreover, due to the use of different mass spectrometry platforms (and thus different lipid classes/species and different resolution/coverage), only a small number of lipid species detected in the SHFS were also available in the Bruneck study. As such, many putative lipids identified in American Indian people were unable to be tested in European White people. In this regard, lipids detected in both populations should assure us of the robustness of the findings. Those identified in 1 but not the other could be attributed to the differences between the 2 populations (eg, demographics, genetics, lifestyle, environmental exposures). Second, our findings were derived from American Indian people with high prevalence of obesity and type 2 diabetes. However, all our analyses adjusted for BMI and diabetes. Moreover, given the rising tides of obesity and diabetes in other racial and ethnic groups worldwide, our findings should be able to generalize to other population settings. Third, although our statistical models controlled many known clinical factors, we cannot exclude the possibility of residual confounding by unknown or unmeasured factors. Finally, as with all other observational studies, we cannot determine whether the observed associations are causal relationship.

However, our study has several strengths. First, the longitudinal profiling of plasma lipidome in a large community-based prospective cohort represents the major strength of this study. Second, the current analysis included ≈2500 participants in 2 community-based prospective cohorts comprising individuals with diverse backgrounds in demographic (eg, age, sex, and socioeconomic status), genetic, lifestyle (eg, diet, and physical activity), and environmental exposures. Despite these differences, many identified lipids could be replicated in both cohorts, signifying the robustness of our findings. Third, compared with previous studies using a targeted metabolomic approach, we used high-resolution liquid chromatography mass spectrometry to profile the fasting plasma lipidome. The high coverage lipidome allowed us to identify novel molecular lipids associated with CHD and offers unprecedented opportunities for future research. Fourth, our statistical analyses adjusted for a comprehensive list of conventional cardiovascular risk factors. Moreover, we performed sensitivity analyses to examine the potential effects of clinical lipids (HDL-c and total cholesterol) and use of lipid-lowering drugs on our results. Thus, lipids identified in our study should be independent of these risk factors. If validated, the newly identified lipid species may have clinical utility in risk stratification, early detection, or prevention of CVD.

In summary, we identified multiple individual lipid species associated with risk of CHD in American Indian people and some were confirmed in European White persons. Our results shed light on the role of dyslipidemia in CHD development and, if validated, may help identify high-risk individuals and offer opportunities for early prevention or intervention of CVD.

ARTICLE INFORMATION

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Affiliations

Department of Epidemiology, College of Public Health & Health Professions and College of Medicine (G.M., J.Z.) and Center for Genetic Epidemiology and Bioinformatics (G.M., J.Z.), University of Florida, Gainesville, FLDepartment of Neurology, Medical University Innsbruck, Innsbruck, Austria (R.P., J.W., S.K.); West Coast Metabolomics Center, University of California, Davis, CA (O.F.); Department of Biostatistics and Epidemiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK (K.M.M., Y.Z.); MedStar Health Research Institute, Hyattsville, MD (J.G.U.); National Heart & Lung Institute, Imperial College, London, UK (M.M.); and Research Centre on Vascular Ageing and Stroke, Innsbruck, Austria (S.K.).

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Disclosures

None.

Supplemental Material

Tables S1–S4
Figures S1–S2

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